Eradication of Resistant Biofilm Forming Medical Device Related Pathogens by Ultrashort Self-assembled Peptide Nanomaterials

Laverty, G., McCloskey, A., Gilmore, B., Zhou, J., & Xu, B. (2015). *Eradication of Resistant Biofilm Forming Medical Device Related Pathogens by Ultrashort Self-assembled Peptide Nanomaterials*. Paper presented at Nanopeptide 2015, Glasgow, United Kingdom. http://www.nanopeptide2015.org/programme-abstracts

Eradication of Resistant Biofilm Forming Medical Device Related Pathogens by Ultrashort Self-assembled Peptide Nanomaterials

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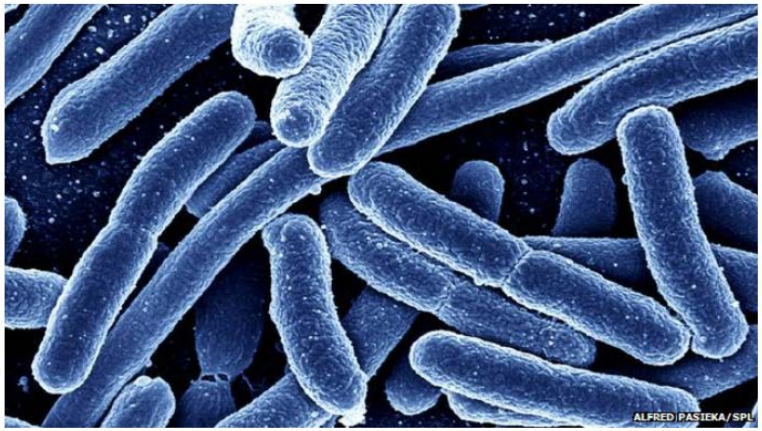
Biochemical Society
Advancing Molecular Bioscience

Antimicrobial Resistance

BBC NEWS

Superbugs to kill 'more than cancer' by 2050

COMMENTS (565)



Drug resistant E.coli bacteria are already a significant problem in Europe

Drug resistant infections will kill an extra 10 million people a year worldwide - more than currently die from cancer - by 2050 unless action is taken, a study says.

They are currently implicated in 700,000 deaths each year.

Related Stories

Analysis: Antibiotic apocalypse

sky NEWS

- Medical device related infections
- Increased reservoir of “superbugs”
- Persistent burden on:
  - Patient morbidity& mortality
  - Family and carers
  - Healthcare budgets

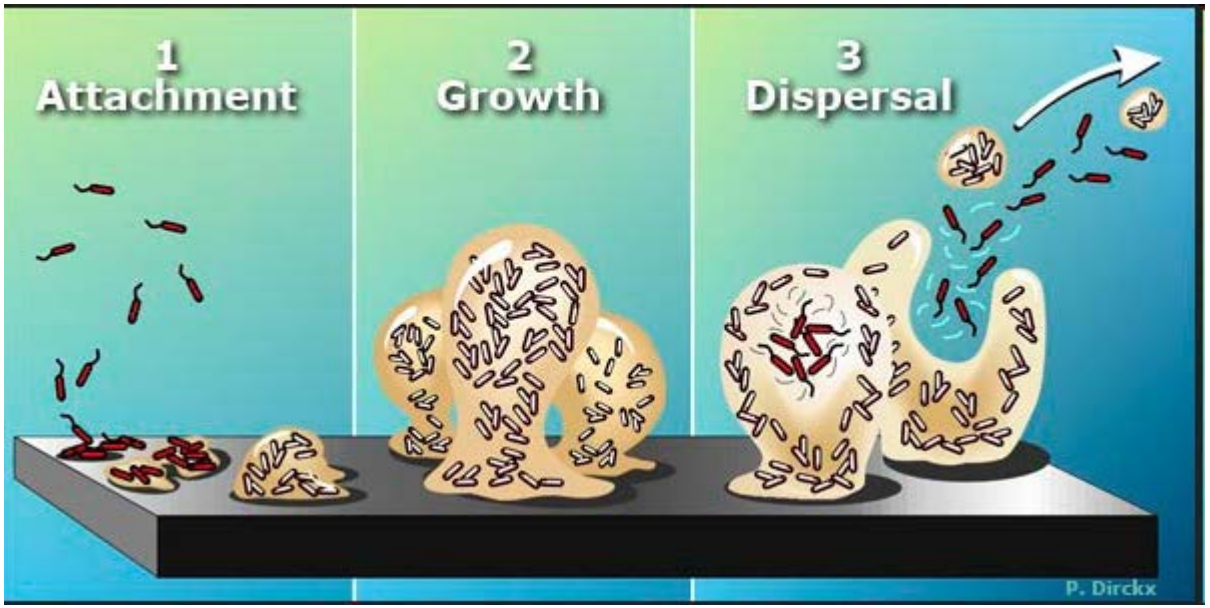
Superbugs 'Could Send UK Back To The Dark Ages'

Action is needed to stop the world entering a post-antibiotic era in which common infections and injuries can kill, say experts.

17:49, UK,
Wednesday 02 July 2014

Planktonic vs. Biofilm Bacteria

- Planktonic form: Free floating in liquid
- Biofilm form: sessile, composed of aggregated microcolonies of cells surrounded by a protective extracellular polymeric matrix
- Mature biofilms can resist 10-1000 times the concentrations of standard antibiotic regimens that are required to kill genetically equivalent planktonic forms



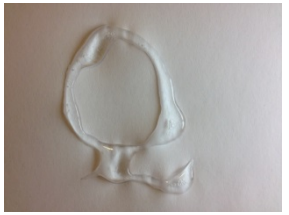
P. Dirckx, Centre for Biofilm Engineering, Montana State University, Bozeman

Biofilms and Implant-Associated Infections. Laverty, G., Gorman, S.P. and Gilmore, B.F. In: Biomaterials and Medical Device Associated Infections. Woodhead Publishing Ltd. 2014.

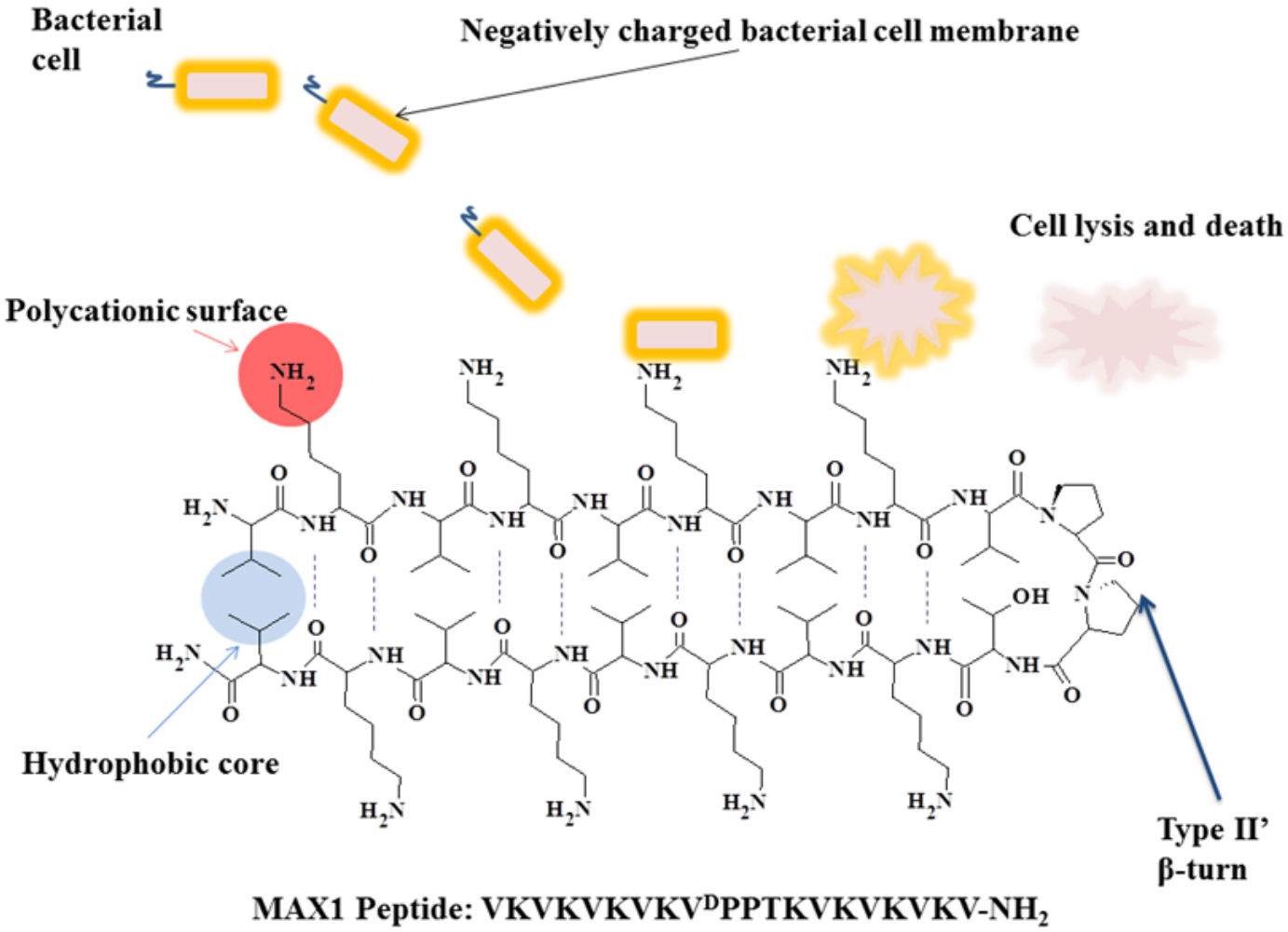
Rational Design of Antimicrobial Peptide Motif vs Self-assembly

Antimicrobial Activity	Propensity to Self-assembled hydrogels
Hydrophobic/Hydrophilic (Charge) ratio (more important with regard to antimicrobial activity than size)	**Hydrophobic/Hydrophilic balance**
Interactions with microbial extracelluar membranes	Non Covalent intermolecular interactions (e.g. Van der Waal's, π-π stacking)
Interaction with intracellular targets/processes (DNA, RNA, enzymes, protein synthesis)	Ability of peptide to form hydrogen bonds with each other and with water

McCloskey A.P., Gilmore, B.F., Laverty, G. (2014) Evolution of Antimicrobial Peptides to Self-Assembled Peptides for Biomaterial Applications. *Pathogens*. *3*(4); 791-821.



Microbiological Applications

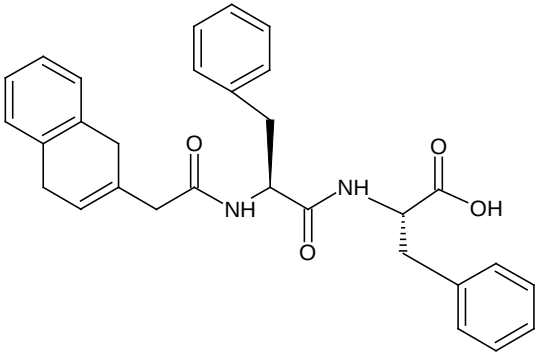


- McCloskey A.P., Gilmore, B.F., Laverty, G. (2014) Evolution of Antimicrobial Peptides to Self-Assembled Peptides for Biomaterial Applications. *Pathogens*. *3*(4); 791-821.
- Schneider, J. P.; Pochan, D. J.; Ozbas, B.; Rajagopal, K.; Pakstis, L.; Kretsinger, J. (2002) Responsive hydrogels from the intramolecular folding and self-assembly of a designed peptide. J. Am. Chem. Soc., 124, 15030-15037.

Self-assembled Ultrashort Peptide Gels

- 2013 Research Placement Prof. Bing Xu Lab, School of Chemistry, Brandeis, Waltham, Boston
- Successful in producing a series of ultrashort peptides (< 7 amino acids) that self-assembled at physiological pH
- More cost effective
- Hydrophobicity provided by inclusion of a naphthalene (Nap) grouping and varying quantity of phenylalanine in primary structure

NapFF structure



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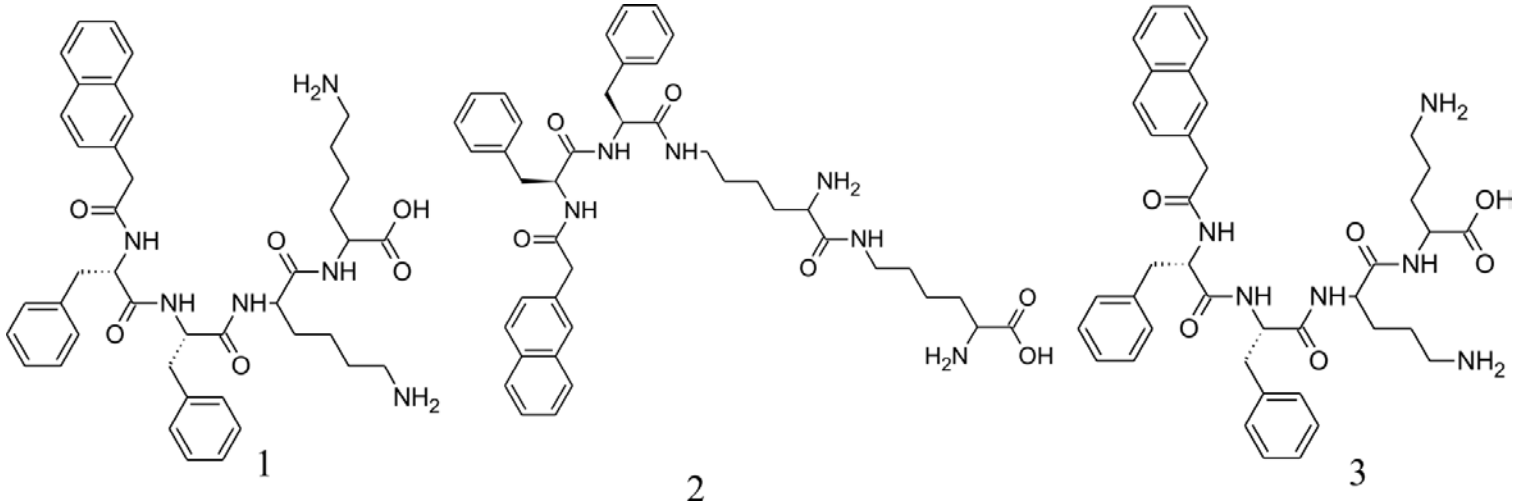
Ultrashort Cationic Variants: Primary Structures

- Charge: Inclusion of cationic amino acids
  - 1) Lysine
  - 2) Ornithine
  - 3) epsilon (ε) Lysine
- Minimum of 2 charged units required for antimicrobial activity
- Primary amine group provides cationic charge
- Cationic amino acids vary by number of methylene units on R-group

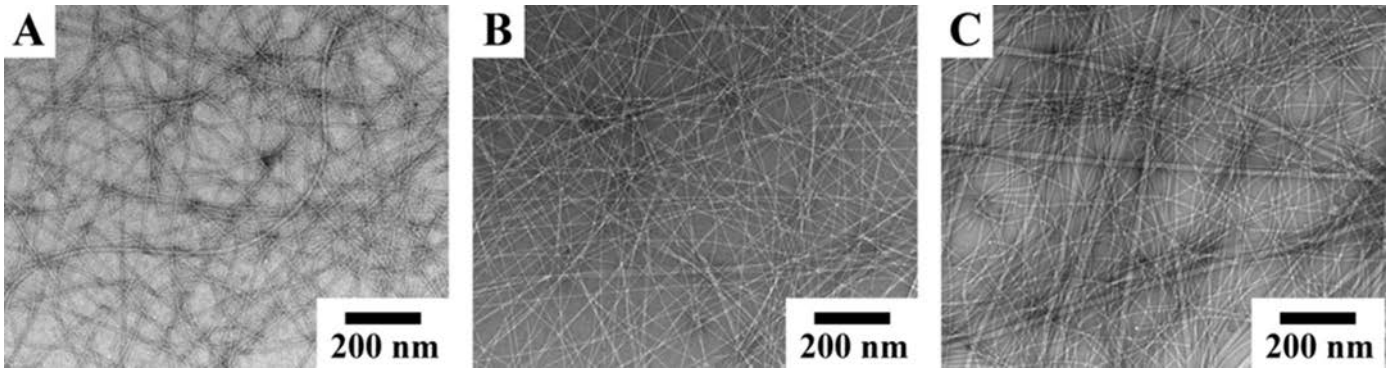


-Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). Ultrashort Cationic Naphthalene derived Self-assembled Peptides as Antimicrobial Nanomaterials. *Biomacromolecules*; 15: 3429−3439.
-Laverty, G., Gorman, S.P. and Gilmore, B.F (2012). The Adherence of *Staphylococcus epidermidis* to Antimicrobial Peptide Incorporated poly(2-hydroxyethyl methacrylate) Hydrogels. *Journal of Biomedical Materials Research: Part A* 100A; 1803–1814.

Ultrashort Cationic Variants: Self-assembly

- Form Self supporting hydrogels at pH 7.4: pH triggering method

Optical images of gel (A) NapFFOO, (B) NapFFKK, (C) NapFFεKεK, at a concentration of 1% w/v and pH of 7.4 in water



Transmission electron microscopy (TEM) images of (A) NapFFOO, (B) NapFFKK, (C) NapFFεKεK, at a concentration of 1% w/v and pH of 7.4 in water

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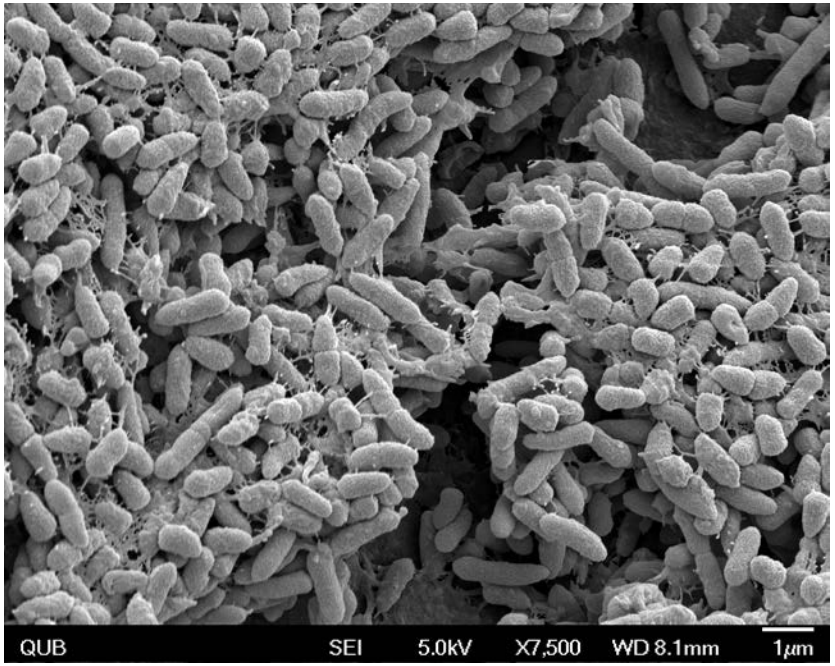
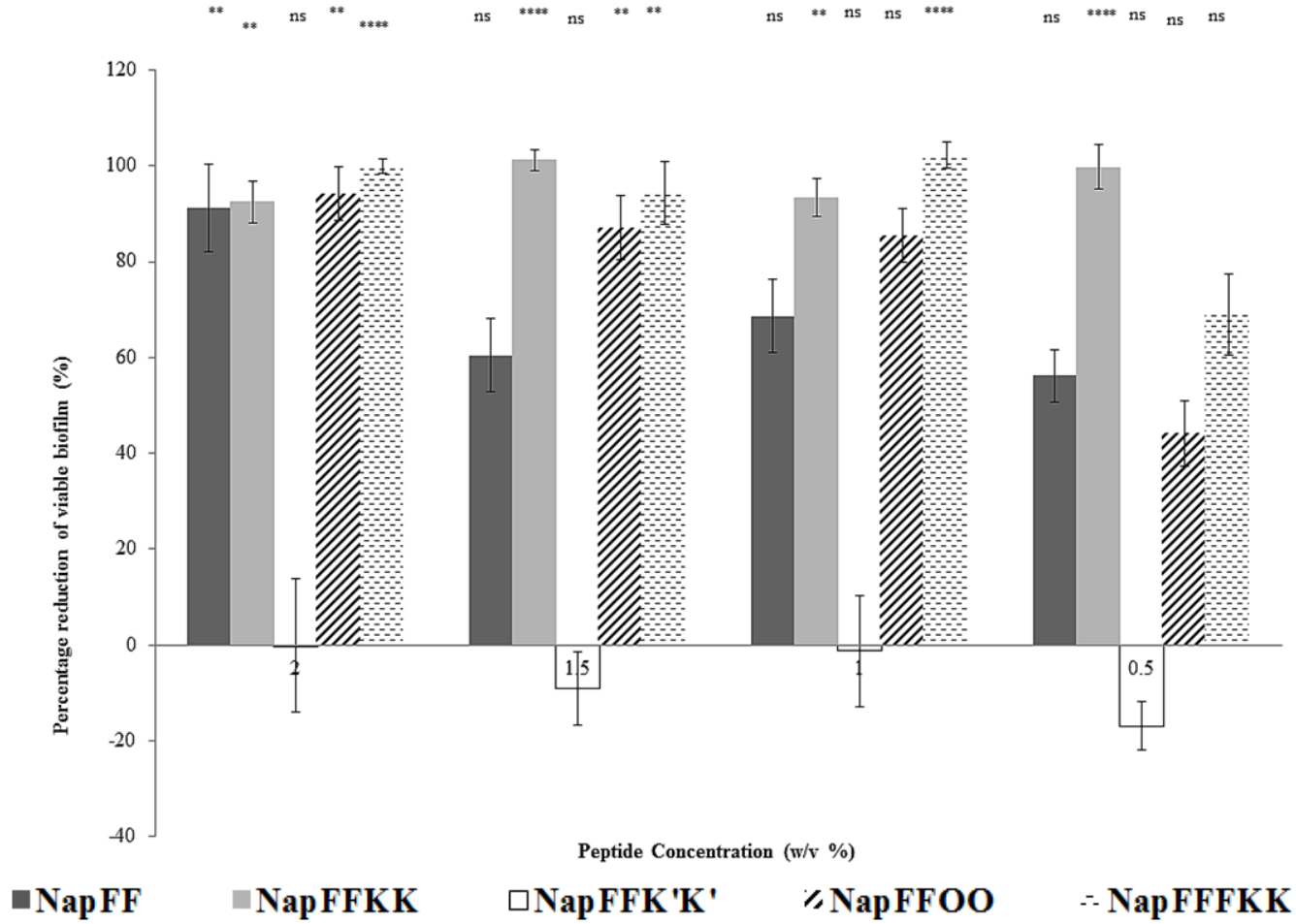
Anti-Biofilm Activity

- alamarBlue Cell Viability assay: 24 hour grown Biofilms
- Gram-positive
  - *Staphylococcus epidermidis* (ATCC 35984)
  - *Staphylococcus aureus* (ATCC 29213)
- Gram-negative
  - *Pseudomonas aeruginosa* (PAO1)
  - *Escherichia coli* (NCTC 11303)
- Medical device related pathogens



SEM *Pseudomonas aeruginosa* (PAO1) attached to catheter surface

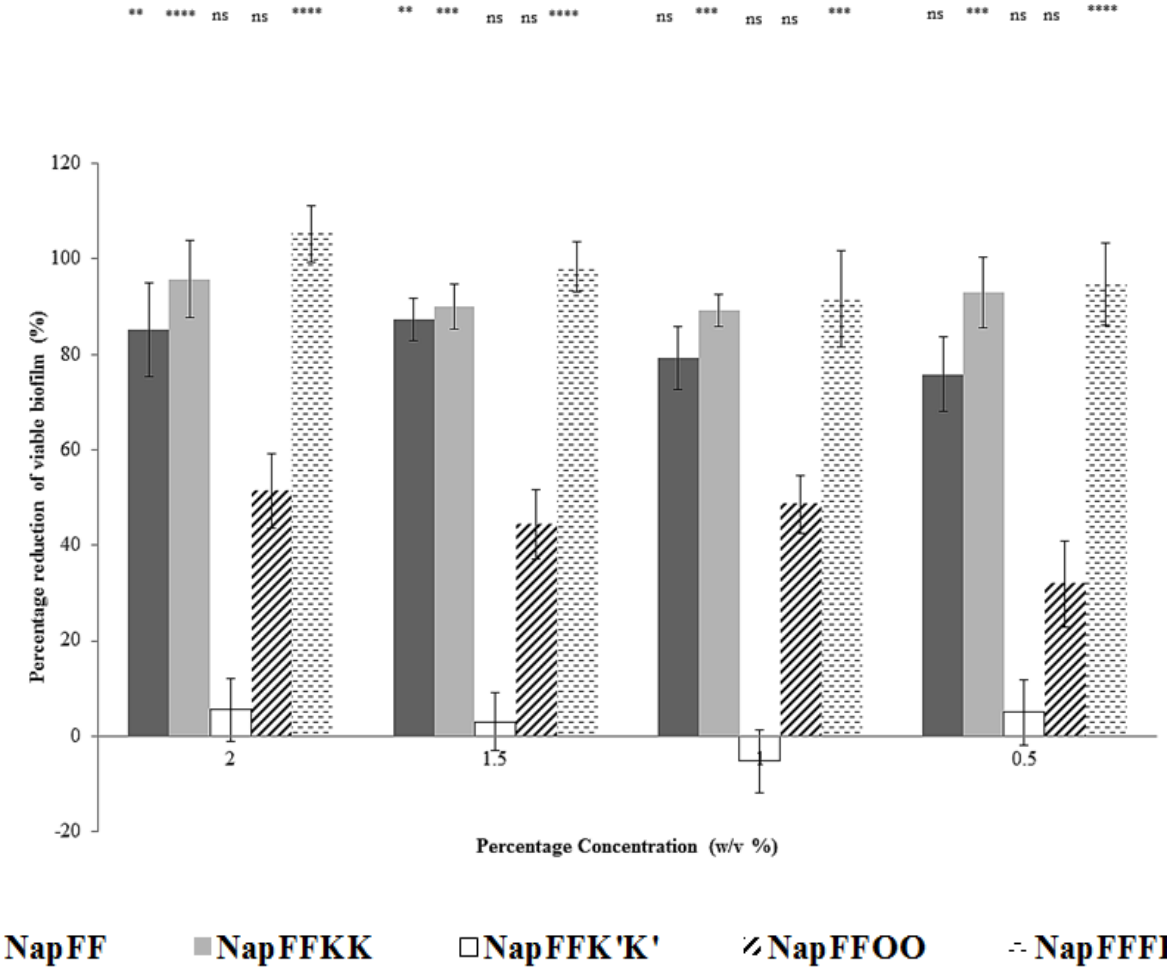
Anti-Biofilm Activity Gram-positive Bacteria



Percentage reduction of mature 24 hour *Staphylococcus aureus* (ATCC 29213) biofilm after 24 hour incubation with naphthalene derived self-assembled hydrogels utilizing an alamarBlue assay. Results are displayed as a mean of 8 replicates

Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). *Biomacromolecules*; **15**: 3429−3439.

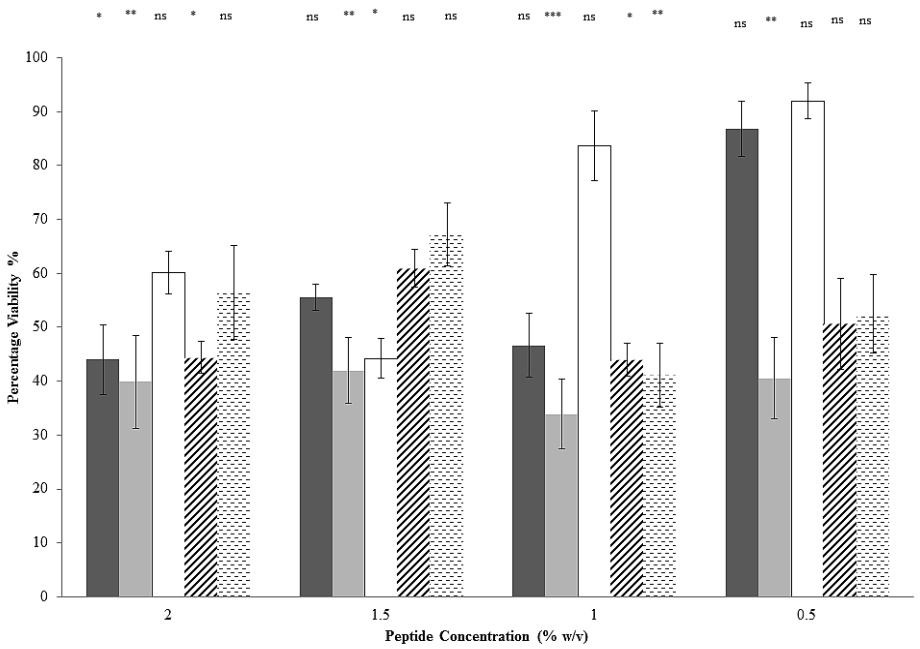
Anti-Biofilm Activity Gram-negative Bacteria

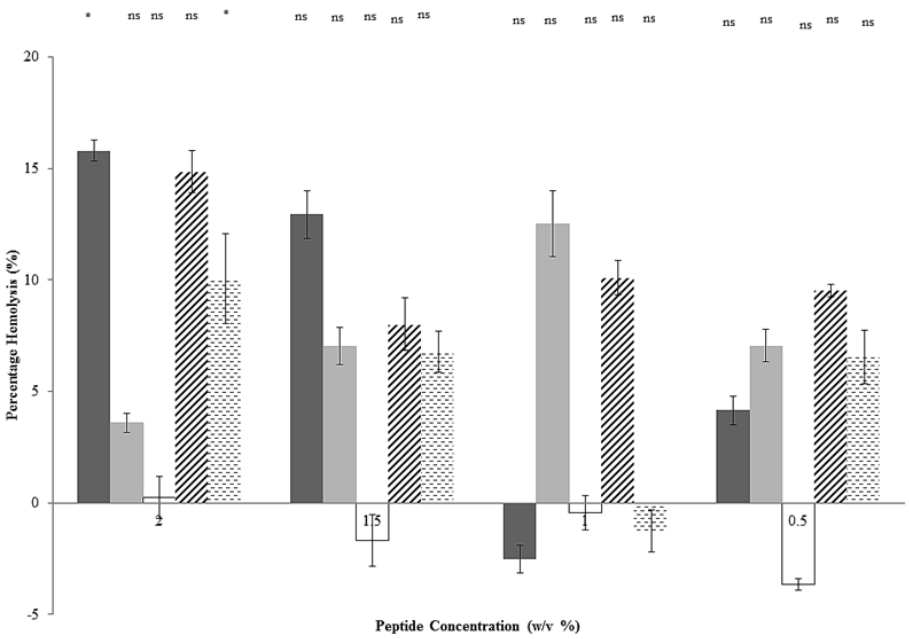


Percentage reduction of mature 24 hour *Escherichia coli* (NCTC 11303) biofilm after 24 hour incubation with naphthalene derived self-assembled hydrogels utilizing an alamarBlue assay. Results are displayed as a mean of 8 replicates

Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). *Biomacromolecules*; 15: 3429−3439.

Toxicity: Tissue Culture & Haemolysis





Percentage viability of CCL 1 [NCTC clone 929]-murine fibroblasts subcutaneous connective tissue monolayer cells after 24 hour exposure to naphthalene derived self-assembled hydrogels utilizing an alamarBlue assay. Results are displayed as a mean of 8 replicates

Percentage hemolysis of the naphthalene peptides against equine erythrocytes. Each value is expressed as the mean of six replicates, incubated at 37 °C for 1 hour

NapFF NapFFKK NapFFK'K' NapFFOO NapFFFKK

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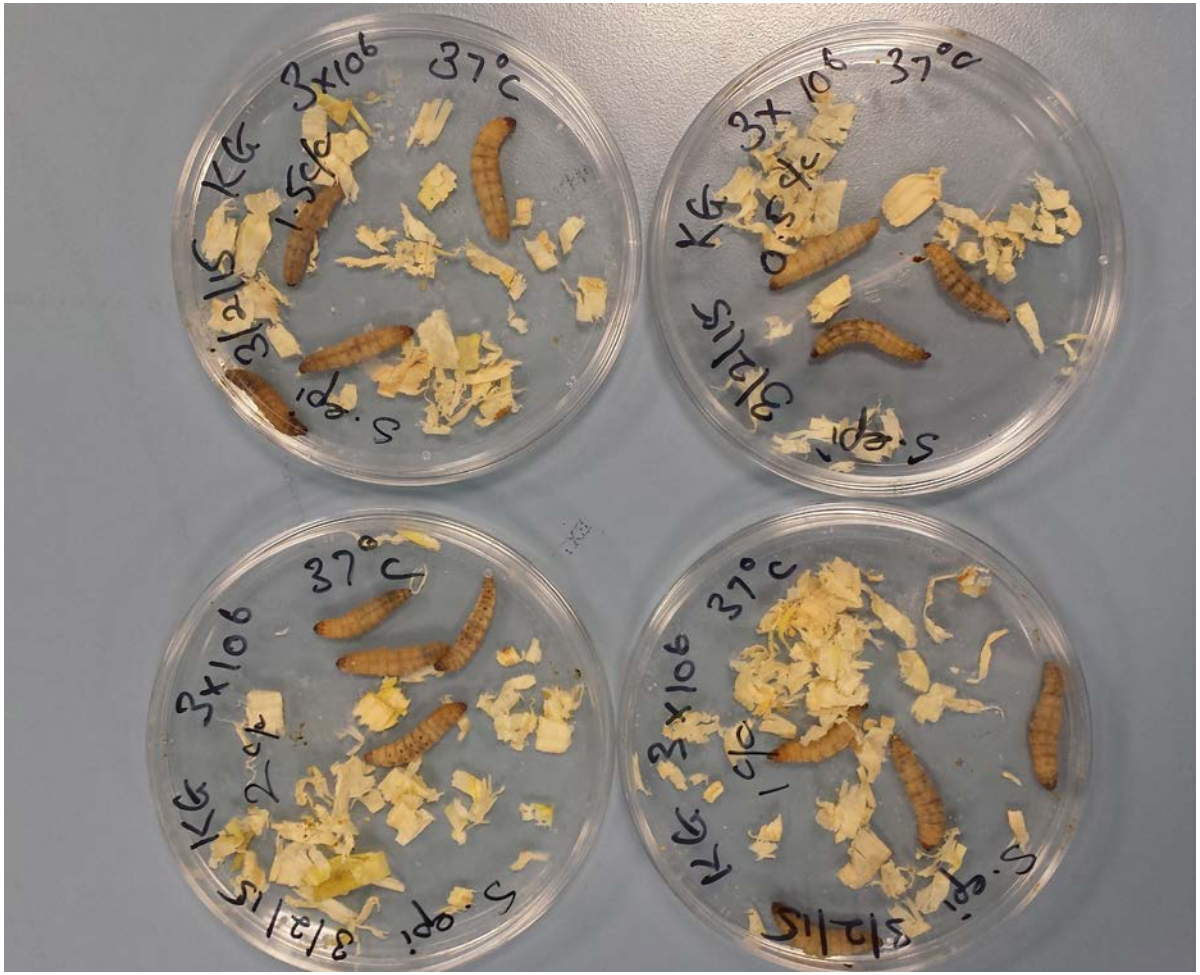
Galleria mellonella (Waxworm) assay

National Centre for the Replacement Refinement & Reduction of Animals in Research



Non viable *Galleria mellonella*

Preliminary data demonstrates biocompatibility (NapFFKK) and reduction in bacterial load with *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (PAO1)

Conclusions

- Greater selectivity was shown against biofilm bacteria compared with mammalian cells
- Selective targeting of negatively charged bacterial membranes by primary amine on cationic amino acid
- The lysine containing ultrashort cationic naphthalene peptides show particular promise
- Antimicrobial activity is related to length of methylene chain on R-group and viability of primary amine
- Further research is required to permit gelation and antimicrobial activity in response to the development of bacterial environmental stimuli *in vivo*
- Thus ensuring antimicrobial activity occurs when it is most required and limiting side effects to patients

Thank You!

- Alice McCloskey (DEL funded PhD student)
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- Sophie Watterson (Nuffield)
- Dr Brendan Gilmore (QUB academic mentor)

- The Xu Group, School of Chemistry, Brandeis.